

Note

Isolation and characterization of novel esters from aerial parts of *Tiliacora acuminata*

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Two novel esters octyl(benzoylamino)acetate and heptadeca-4-ene-acetate have been isolated from the aerial parts of both male and female plants of *Tiliacora acuminata*. The structures are confirmed by spectroscopic and analytical methods.

Keywords: *Tiliacora acuminata*, octyl(benzoylamino)acetate, heptadeca-4-ene-acetate

The extracts of *Tiliacora acuminata* are used in many Ayurvedic preparations and regarded as an antidote for snakebite¹. A new lactone², two alkaloids tiliaresine³ and (+) *N*-methyltiliamosine⁴ from leaves and an oil acuminatide⁵ from seeds have been isolated and characterized. In continuation of earlier work the isolation and characterization of two novel esters from the aerial parts of both male and female plants of *Tiliacora acuminata* are reported.

Results and Discussion

The dried aerial parts of the *Tiliacora acuminata* were extracted using Soxhlet with chloroform for eight hr. The same process was repeated four times. Finally the extracts were combined and filtered. The extract was concentrated under ordinary distillation and column chromatographed over silica gel. It was eluted with hexane and after evaporation, an oily liquid **1** was obtained. The oil was further purified on preparative TLC using a solvent mixture of hexane-ethylalcohol in the ratio 9:1. The column was further eluted with chloroform-ethanol (9:1 v/v) and after evaporation of the solvent gave another oily compound **2**.

Compound **1** was highly soluble in diethylether, chloroform, benzene and ethylalcohol and insoluble in 10% sodium bicarbonate and sodium hydroxide solutions. It decolourised Baeyers reagent indicating unsaturation in the molecule. The reaction with 5% sodium hydroxide at hot condition showed the

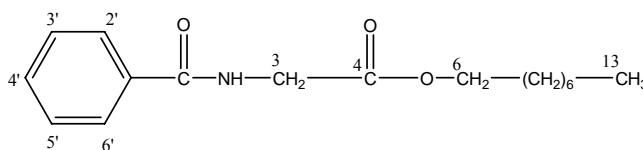
presence of ester functionality and the separation of solid after acidification gave effervescence with 10% sodium bicarbonate solution. The CHN analysis indicated the presence of hippuric acid moiety^{6,7}

The carbon-hydrogen analysis and the M^+ peak in the mass spectrum at 292.2 favoured the molecular formula $C_{17}H_{25}O_3N$. The intense UV absorption at 200 nm was attributable to the $n-\pi^*$ transition of the carbonyl group and the other values at 210, 230 and 250 nm correlated to the presence of benzene ring⁸. The IR band at 3369 cm^{-1} indicated the presence of N-H group and the two other prominent peaks at 1719 and 1650 cm^{-1} confirmed the presence of two carbonyl groups⁹. The C–O–C and C–N vibrations were observed at 1026 and 1111, 1276 cm^{-1} respectively¹⁰. The three bands at 1451, 1489 and 1537 cm^{-1} favoured the presence of benzene ring¹⁰.

The ^1H NMR spectrum (**Table I**) revealed the presence of $-\text{CH}_3$ protons (δ 0.9) as a triplet and the twelve $-\text{CH}_2$ -protons δ 1.2 to 1.6 appeared as a broad singlet (H-7 to H-12). The proton integration of the above two sets accounted for 15 protons. The other $-\text{CH}_2$ proton (H-3) appeared at δ 3.4 as a doublet due to deshielding effect of carbonyl and N–H groups¹¹. The peak for N–H proton appeared as broad singlet at δ 6.7. The $-\text{CH}_2-$ proton (H-6) near to carboxyl group showed triplet at δ 4.3 due to deshielding nature of oxygen. The two aromatic protons (H-2', 6') near to carbonyl of the side chain appeared as a doublet (δ 8.05 to 8.15) with *J* value 6 Hz and a doublet at (δ 7.85) respectively¹². The other three protons were present as multiplet between δ 7.4 and 7.7.

The proton decoupled ^{13}C NMR spectrum revealed the presence of carbonyl groups at δ 167.6 and 170.1 (C-1, 4). The carbon of the methylene group adjacent to oxygen atom appeared at δ 64.9 (C-6) and the other methylene group adjacent to oxygen atom appeared at δ 41.9 (C-3). The six aromatic carbons were resolved at δ 133.1 (C-1') and 128.3 (C-2'), 132.6, 132.4, 129.7 (C-3' to 5') and 127 (C-6').

The mass spectral fragment at *m/z* 180 indicated strongly the presence of hippuric acid moiety and the other fragment ion at *m/z* 123 confirmed the position of N–H group. The *m/z* values at 224 and 278 favoured the long chain alkane moiety.

Table I — ^1H and ^{13}C NMR characterization data of compound **1**

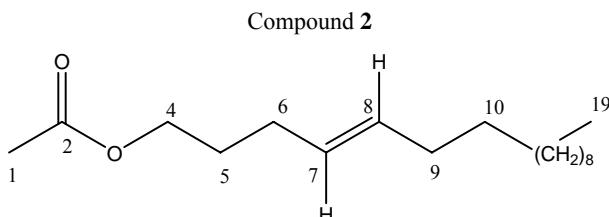
Carbon number*	^1H NMR (δ , ppm)	^{13}C NMR (δ , ppm)
1	—	167.6
3	3.4 d	41.9
4	—	170.1
6	4.3 t	64.9
7-12	1.2 to 1.6 brs	35.6, 31.5, 28.9, 25.0, 25.5, 22.3
13	0.9 t	13.8
1'	—	133.1
2'	8.05 to 8.15 d (6 Hz)	128.3
3'-5'	7.4-7.7 m	132.6, 132.4, 129.7
6'	7.85 d	127
N-H	6.7 brs	—
CDCl ₃ peek	7.2	76.6, 76.9, 77.3

*Numbering as shown in structure of compound **1**
(CDCl₃, ^1H : 400 MHz, ^{13}C : 100 MHz), J values are in parentheses

The compound **1** isolated from male plant of *Tiliacora acuminata* favoured similar R_f value, CHN analysis, qualitative tests and the formation of hippuric acid moiety during the ester hydrolysis.

The compound **2** was soluble in chloroform, diethyl ether, ethanol and methanol. The positive reaction with bromine in CCl₄ and dilute potassium permanganate solution showed the presence of unsaturation. The compound failed to respond 2,4-dinitrophenylhydrazine but positive with sodium hydroxide-phenolphthalein indicator test confirming the presence of ester functionality. On the basis of mass spectrum and carbon-hydrogen analysis the molecular formula of the compound was found to be C₁₈ H₃₄ O₂ (M⁺ peak at *m/z* 282).

The chemical shift values (**Table II**) of the methyl protons adjacent to carbonyl group appeared as a broad singlet at δ 1.7 and the same was observed in proton decoupled ^{13}C NMR spectrum at δ 22.5 (Ref. 13). The methylene protons near to the oxygen of the ester linkage splitted by the nearby protons and appeared as triplet (δ 4.12 to 4.29). In ^{13}C NMR spectrum the same methylene carbon was present at

Table II — ^1H and ^{13}C NMR characterization data of

Carbon number*	^1H NMR (δ , ppm)	^{13}C NMR (δ , ppm)
1	1.7 s	22.5
2	—	173.12
4	4.12 to 4.29 t	68.9
5	1.6 brs	31.9
6	2.1 dt (12, 7 Hz)	33.6
7-8	5.3-5.4 m	129.6, 130.5
9	2.1 dt	34.1
10	1.6 brs	31.5
11-18	1.3 brs	29.6, 29.4, 29.3, 28.9, 28.2, 24.8
19	0.9 t	14.02
CDCl ₃ peek	7.2	76.6, 76.9, 77.3

*Numbering as shown in structure of compound **2** (CDCl₃, ^1H : 400 MHz, ^{13}C : 100 MHz), J values are in parentheses

δ 68.9. The methylene protons adjacent to carbon-carbon double bond were found to be at δ 2.1 (dt 12, 7 Hz). The remaining methylene protons were appeared as broad singlets at δ 1.6 and 1.3. The methyl protons appeared as triplet at δ 0.9. The presence of an isolated double bond was exhibited by multiple resonances due to olefinic protons at δ 5.3-5.4 (H-7, H-8). The two carbon peaks of double bond were exhibited in ^{13}C NMR spectrum at δ 130.5 (C-7) and 129.6 (C-8). The configuration of the carbon-carbon double bond was suspected to be trans based on the chemical shift of adjacent carbons in the ^{13}C NMR data (δ 33.6, 34.1). Usually the signals of carbon next to a double bond appear at δ 27-28 in *cis* configuration while those of *trans* configuration appear at δ 32-34 (Ref. 14, 15).

The fragment ion peak of compound **2** at *m/z* 114 was indicative of double bond between C-7 and C-8. The presence of other fragment ions at *m/z* 197, 225 suggested the long chain alkane moiety.

The compound **2** isolated from male plant of *Tiliacora acuminata* using the same procedure showed similar analytical data solubility, aliphatic nature, ester functionality and the reaction with periodic acid test.

Experimental Section

Plant materials were air dried for twenty days. The Soxhlet extractor was used for the extraction process for female and male plants of *Tiliacora acuminata*. Toshniwal capillary melting point apparatus was used for the determination of melting point. The UV-vis spectra were taken in a Varian-Cary spectrometer and methanol was the solvent. IR spectra were recorded in a Brucker IF6-66 V FT-IR spectrometer. The ¹H and ¹³C NMR were recorded in AMX 400 instrument and the frequencies used were 400 MHz and 100 MHz respectively.

Octyl(benzoylamino)acetate **1**

(Female Plant) compound 1: C₁₇H₂₅O₃N (292.2), Calcd: C, 70.07; H, 8.65; N, 4.81. Found: C, 70.15; H, 8.75; N, 4.79%. Hydrolysis reaction with sodium hydroxide yielded solid hippuric acid and its melting point was 82.5°C (reported 83°C). The carbon-hydrogen analysis of hippuric acid was identical with the literature (Ref. 6, 7).

(Male Plant) Anal. C, 70.23; H, 8.83; N, 4.74. R_f = 0.92. Hydrolysis reaction with alcoholic potassium hydroxide yielded hippuric acid moiety.

Hippuric acid reacted with thionyl chloride¹⁶ in methanol indicating the monomethylation of carboxyl group.

Heptadeca-4-ene-acetate **2**

(Female Plant) R_f = 0.75. Analysis for C₁₈H₃₄O₂ (282), Calcd: C, 76.53; H, 12.04; Found C, 76.91;

H, 12.12. UV-Vis: 200 (nonconjugated C=C), 230 nm ($\pi-\pi^*$ of CO); IR: 1746 (–COO), 1265 (C–O), 2924, 2854 (C–H_{str}), 902, 735 cm⁻¹ (disubstituted C=C).

(Male Plant) R_f = 0.74, Reaction with periodic acid¹⁷ in the presence of ruthenium chloride gave dodecanoic acid and verified with melting point (observed 44°C, reported 43°C). It indicated 12 carbons in alkane moiety after the double bond.

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